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14. ABSTRACT

WASF3 functions as a key regulator in controlling metastasis of prostate cancer and inhibiting it prevents metastasis. There are no drugs available to target WASF3 directly, but there are many FDA approved drugs target the other proteins that it interacts with. Using preclinical animal models of prostate cancer, we aim to investigate whether the proposed drugs that target WASF3-dependent pathways can be repurposed in an attempt to suppress prostate cancer metastasis. The major achievement of our research in the first year of this funding is: 1) have screened the effects of more than 40 drugs on invasion using cultured prostate cancer cells and found that targeting multiple points in WASF3 regulatory network by co-treatment was more efficient to suppress invasion than either drug alone; 2) we have established drug toxicity and the effective dose in zebrafish and found the best performing strategies using zebrafish-metastasis models. This has allowed us to transfer the findings to in vivo mouse metastasis assays. If success, it provides a great means to improve the quality of life for metastatic prostate cancer patients.

15. SUBJECT TERMS

Prostate cancer, metastasis, WASF3, drug screen, zebrafish, mouse, therapies

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Introduction

Death due to prostate cancer results largely from metastatic spread of the disease. Defining novel gene and pathways that lead to metastatic progression will expand opportunity to provide systemic adjuvant therapies during the early stages to limit the spread of cancer. One such pathway involves overexpression of the WASF3 gene which promotes prostate cancer metastasis and inactivation of this gene by genetic approaches can suppress invasion and metastasis of prostate cancer cells. Although there are no drugs that target WASF3 directly, WASF3 regulates many pathways and targeting critical nodes in these pathways using genetic approaches can suppress metastasis. Drugs are also available to target the same nodes in these pathways but these drugs have not been considered in the context of metastasis. Using both zebrafish-metastasis models and mouse models of prostate cancer, we aim to investigate whether FDA approved drugs that target these pathways can be repurposed in an attempt to suppress prostate cancer metastasis. We believe that by suppressing WASF3-dependent metastasis or significantly delaying it will lead to improvement in overall survival and improve the quality of life for the patient. This proposal will provide the preclinical data to facilitate trials in this area.

Keywords

Prostate cancer, metastasis, WASF3, drug screen, zebrafish, mouse, therapies

Accomplishments

1. What were the major goals and objectives of the project?

The goal of our research in the first year of this funding is to 1) establish zebrafish-metastasis models and drug screen using zebrafish (month 1-9) and to 2) analysis zebrafish data (month 10-12).

2. What was accomplished under these goals?

To achieve this goal, we cultured high-invasive prostate cancer PC3 cells and treated them with the drugs/inhibitors that were proposed to target WASF3-dependent molecular pathways. We then performed Transwell invasion assays (BD) to analyze the individual drug effect on invasion suppression. As shown in Fig. 1, after 24 hours incubation, 10 µM Dasatinib and 10 µM CYT997

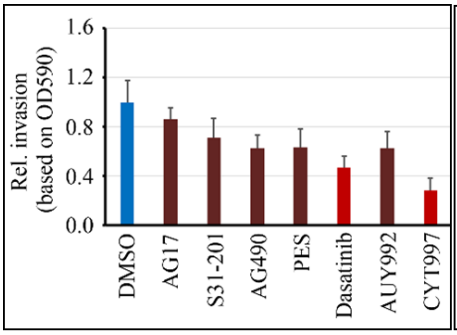

Fig. 1 Summary of the effects of a few representative drugs on prostate cancer cell invasion. Matrigel invasion assays were performed using transwells (BD biosciences, CA) with 8-µm pore size filters. Briefly, 25000 cells were placed in the upper chamber and RPMI medium containing 1% FBS with different drugs (such as PDGF inhibitor AG17, STAT3 inhibitor S31-201, JAK2 inhibitor AG490, HSP70 inhibitor PES, SRC inhibitor Dasatinib, HSP90 inhibitor AUY992 and tubulin inhibitor CYT997) was added to the lower chamber. After 24 hours incubation, invading cells were stained with crystal violet and eluted with 10% acetic acid extraction buffer. The absorbance was calculated at 590 nm in each well and each sample was analyzed in triplicate.

inhibited cell invasion more efficiently than other drugs/inhibitors. We further investigated the combined effects of these two drugs on WASF3 function and the consequence of cell invasion. Co-inhibiting the activation of SRC (Dasatinib) and Tubulin (CYT997) impaired WASF3 activation and expression and suppressed cell invasion more significantly than either drug alone (Fig. 2). Based on these results, we concluded that the targeting multiple points in the WASF3 regulatory network can prevent prostate cancer invasion and many of the drugs used in this study could act synergistically.

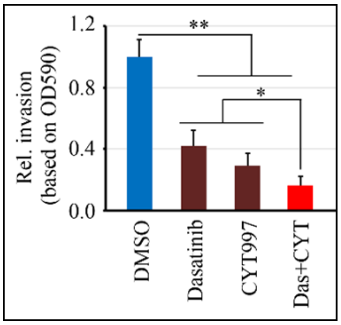

Fig. 2 Co-treatment of Dasatinib (Das) and CYT997 (CYT) suppressed prostate cancer cell invasion more significantly than either Dasatinib or CYT997 alone. Matrigel invasion assays were performed as described in Fig. 1. All the experiment was repeated independently in triplicate and consistent results were obtained. *P < 0.05 and ** P < 0.01.

Zebrafish genetic and transplant models have emerged as a promising intact vertebrate system for modeling human cancers, which are particularly well suited for whole-organism anti-cancer drug evaluation. We then translated *in vitro* findings into the novel metastasis model we have developed in zebrafish. In *in vivo* experiment, we firstly established drug toxicity for each inhibitor. More than 20 drugs were subjected to a dose-response assay at ranging from 10 nM -1 mM in 2-day old zebrafish. Zebrafish larvae were maintained in 24-well plate and examined after 4 days of incubation in drugs. Zebrafish toxicity studies showed that most drugs (such as PES) had significantly high toxicities (abnormal development or death) at the dose<5 µM, while 6 drugs, including CYT997 (100 µM) and Dasatinib (10 µM), have no obvious larval toxicity in the treatment (Fig. 3). These drugs were retained as candidates for further efficacy evaluation. We next tested whether these drugs affected human cancer cell metastasis in zebrafish. We developed the zebrafish metastasis models by injecting ~200 Dil-CM labeled PC3 cells into 2-day old

embryos. The injected zebrafish were returned to water containing the various matched drugs and maintained at 34°C. The fresh drugs at the effective dose were added directly into water every 48 hours for two times before analysis. We then used florescent microscopy to quickly screen all the fish to identify phenotype (metastasis) after drug-treatment within 4 days. No obvious side-effect was observed in fish in the presence of the drugs. Percentage of metastasis was set as the number of embryos containing more than 5 cells outside the yolk sac. Total metastasis percentage was set as the total number of embryos with metastasis at 4 days post injection relative to day zero. Compared with the control groups (treated by DMSO), either treated by 100 μM CYT997 or 10 μM Dasatinib suppressed the cells to spread throughout the fish body (Fig. 4). As expected, co-treatment CYT997 and Dasatinib suppressed cell metastasis more efficiently than either drug alone (Fig. 4). These observations suggest that CYT997 combined with Dasatinib may represent a novel therapeutics for metastatic prostate cancer.

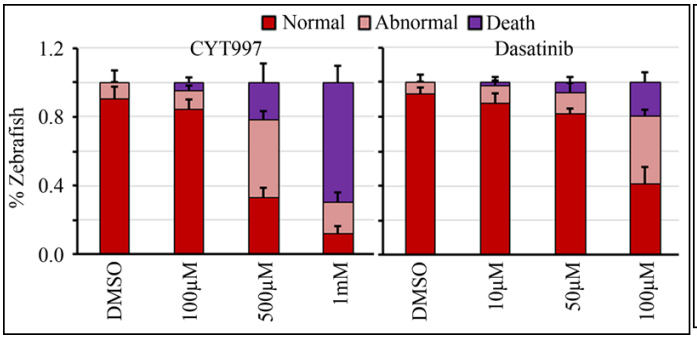

Fig. 3 Establishment of the effective dose and drug toxicity in zebrafish. Initially, 2-day old zebrafish were treated with doses of drugs ranging from 10 nM -1 mM in sets of 10 zebrafish each. Once the effective dose and drug toxicity is established for each drug, the experiment was repeated using 100 embryos at that dose. Only the data of CYT997 and Dasatinib were shown here.

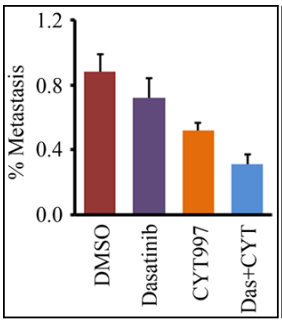

Fig. 4 Co-treatment of Dasatinib (Das) and CYT997 (CYT) suppressed metastasis of prostate cancer cells more efficiently than either drug alone in zebrafish. The in vivo metastasis assays were performed as described in Fig. 3. This experiment was repeated using at least 50 zebrafish per group.

Currently, we have just begun to transfer these findings into mouse models of prostate cancer and aim to use these rodent models to confirm the best performing strategies by the end of second year of the funding. So far, we are on the schedule for our milestone of this project.

3. What opportunities for training and professional development did the project provide?

During this reporting period, I have attended the AACR Annual Meeting 2015 which was held April 18-22 in Philadelphia, Pennsylvania. This meeting highlighted the latest, most exciting discoveries in prostate cancer research and provided a unique opportunity for me to meet, interact, and share my insights with other early-career and established researchers. I present my poster at this meeting and was invited to make presentation at University of New Mexico Cancer Center and University of Toledo.

4. How were the results disseminated to communities of interest?

Nothing to Report.

5. What do you plan to do during the next reporting period to accomplish the goals and objectives?

The goal of our research in the second year of this funding is to validate the drug effects on prostate cancer metastasis in mouse models.

Impact

1. What was the impact on the development of the principal discipline(s) of the project?

We have proven that WASF3 is a very compelling target to limit metastasis in prostate cancer and identified the best performing drugs that inhibited metastasis through targeting multiple points in the WASF3 regulatory network by using zebrafish-metastasis model. Our study during this reporting period has a great significance in the field of "Develop effective treatments for men with high risk or metastatic prostate cancer."

2. What was the impact on other disciplines?

Nothing to Report.

3. What was the impact on technology transfer?

Nothing to Report.

4. What was the impact on society beyond science and technology?

Nothing to Report.

Changes/Problems

Nothing to Report.

Products

Journal publications

Teng Y, Ren X, Li H, Shull A, Kim J, Cowell JK. (2015). Mitochondrial ATAD3A combines with GRP78 to regulate the WASF3 metastasis-promoting protein. Oncogene. *doi: 10.1038/onc.2015.86.*

An acknowledgment of DOD support appeared in this publication.

Participants & Other Collaborating Organizations

1. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

2. What other organizations were involved as partners?

Nothing to Report.

Special Reporting Requirements

None

Appendices

Copy of 1 manuscript rusulting from the research has been submitted to the GOR at Usarmy.detrick.medcom-cdmrp.mbx.cdmrp-reporting@mail.mil

Teng Y, Ren X, Li H, Shull A, Kim J, Cowell JK. (2015). Mitochondrial ATAD3A combines with GRP78 to regulate the WASF3 metastasis-promoting protein. Oncogene. *doi: 10.1038/onc.2015.86.*